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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Zarling, *et al.*	Examiner:	B. Loeb
		Group Art Unit:	1636
Serial No.	09/886,171 (CON of 09/373,347)		
Filed:	June 20, 2001		
For:	*Domain Specific Gene Evolution*		

CERTIFICATE OF MAILING

I hereby certify that this correspondence, including listed enclosures, is being deposited with the United States Postal Service as First Class Mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, DC 20231.

Date: September 20, 2001

Signed: Maria Ciganovich

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

Prior to examination, please amend the above-identified application as follows:

IN THE SPECIFICATION:

Please delete the paragraph on page 1 beginning at line 4 and replace it with the following rewritten paragraph:

> --This application is a continuation of U.S. Application No. 09/373,347, filed August 12, 1999, which is a continuation-in-part of U.S. Application No. 09/133,934, filed August 14, 1998, now U.S. Patent No. 6,074,853, and which claims the benefit of U.S. Provisional Application No. 60/096,330, filed August 12, 1998.—

Please delete the paragraph on page 37 beginning at line 11 and replace it with the following rewritten paragraph:

> To evolve the scFV to higher affinities, probes are synthesized to target CDRs in the light and heavy chains. Each probe has sequences that are dengerate for corresponding CDRs, but homologous to the frame-work regions for homology clamping (Figure 5). The probes are combined with RecA to form nucleoprotein filament as described in Example 1. The filament are hybridized to purified scFv phagemid DNA to produce a hybrid complex. Complexes are transformed into recombination proficient *E. coli* strain (e.g. BB4) to allow strand exchange. The bacteria are also transformed with helper phage to assemble and package the scFv phagement containing mutagenized or evolved CDR regions.

Serial No.: 09/886,171
Filing Date: June 20, 2001

IN THE CLAIMS:

Please amend claims 1-24 as follows:

1. (Amended) A method of domain specific gene evolution of a target nucleic acid encoding an amino acid sequence of interest, said method comprising:

contacting a target nucleic acid with a recombinase and a first plurality of pairs of single-stranded targeting polynucleotides which are substantially complementary to each other, wherein each said targeting polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a first predetermined sequence of said target nucleic acid encoding a first domain of a polypeptide, said first plurality of pairs comprising a first library of nucleic acids having mismatches between said targeting polynucleotides and said first predetermined sequence, to form a first library of altered target nucleic acids; and repeating said contacting on said library of altered nucleic acids.

2. (Amended) A method according to claim 1, further comprising:

contacting said target nucleic acid with a second plurality of pairs of single-stranded targeting polynucleotides which are substantially complementary to each other and are not substantially complementary to said first plurality of polynucleotides, wherein each said targeting polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a second predetermined sequence of said target nucleic acid encoding a second domain of said polypeptide, said second plurality of pairs comprising a second library of nucleic acids having mismatches between said second targeting polynucleotides and said second predetermined sequence, to form a second library of altered target nucleic acids.

3. (Amended) A method of domain specific gene evolution comprising:

a) contacting a target nucleic acid encoding a polypeptide of interest with a recombinase and a first pair of single-stranded targeting polynucleotides which are substantially complementary to each other, wherein each said targeting polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a first predetermined sequence of said nucleic acid encoding a first domain of said polypeptide, to form a first recombination intermediate;

b) contacting said recombination intermediate with a nuclease to form a nicked or open ended target nucleic acid; and

c) reassembling and recombining said nicked or open ended target nucleic acid to evolve a first library of altered target nucleic acids.

4. (Amended) A method according to claim 3 further comprising:

d) combining said target nucleic acid with a second pair of single-stranded targeting polynucleotides which are substantially complementary to each other and are not substantially complementary to said first pair of polynucleotides, wherein each targeting polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a second predetermined sequence of said target nucleic acid encoding a second domain of said polypeptide, to form a second recombination intermediate, wherein said contacting of step b) is of said second recombination intermediate with said nuclease.

5. (Amended) A method of generating a library of altered nucleic acid sequences of a pre-selected target nucleic acid sequence in an extrachromosomal sequence, said method comprising:

a) contacting an extrachromosomal nucleic acid comprising a target nucleic acid sequence with at least one recombinase and a first plurality of pairs of single-stranded targeting polynucleotides which are substantially complementary to each other, wherein

each said targeting polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a first preselected sequence of said target nucleic acid, said first plurality of pairs comprising a first library of nucleic acids having mismatches between said targeting polynucleotides and said first preselected sequence, to evolve a first library of altered target nucleic acids; and

b) repeating step a) on said library of altered target nucleic acids.

6. (Amended) A method according to claim 5 further comprising:

c) adding to said extrachromosomal nucleic acid a second plurality of pairs of single-stranded targeting polynucleotides which are substantially complementary to each other and are not substantially complementary to said first plurality of polynucleotides, wherein each said targeting polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a second preselected sequence of said target nucleic acid, said second plurality of pairs comprising a second library of nucleic acids having mismatches between said targeting polynucleotide and said second preselected sequence, to evolve a second library of altered target nucleic acids,

wherein said repeating is on said second library of altered target nucleic acids.

7. (Amended) A method of generating a library of altered nucleic acids of a pre-selected target nucleic acid in a chromosomal sequence, said method comprising:

a) contacting a chromosomal nucleic acid comprising a target nucleic acid with at least one recombinase and a first plurality of pairs of single-stranded targeting polynucleotides which are substantially complementary to each other, wherein each said polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a first preselected sequence of said target nucleic acid, said plurality of pairs comprising a first library of nucleic acids having mismatches between said targeting polynucleotides and said first preselected sequence, to form a first library of altered target nucleic acids; and

b) repeating step a) on said library of altered target nucleic acids.

8. (Amended) A method according to claim 7 further comprising:

c) adding to said chromosomal nucleic acid a second plurality of pairs of single-stranded targeting polynucleotides which are substantially complementary to each other and are not substantially complementary to said first plurality of polynucleotides, wherein each said polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a second preselected sequence of said target nucleic acid, said second plurality of pairs comprising a second library of nucleic acids having mismatches between said targeting polynucleotides and said second preselected sequence, to evolve a second library of altered target nucleic acids,

wherein said repeating is on said second library of altered target nucleic acids.

9. (Amended) A method according to any one of claims 1, 2, 3, 4, 25, 26, 27, and 28 further comprising repeating said method on said library of altered target nucleic acids.

10. (Amended) A method according to any one of claims 1, 2, 3, 4, 5, 6, 7, 8, 25, 26, 27, and 28 further comprising introducing said library of altered target nucleic acids into cells to form a cellular library comprising variant nucleic acid sequences.

11. (Amended) A method according to claim 10 further comprising expressing said library of altered target nucleic acids to generate a library of variant polypeptides.

12. (Amended) A method according to claim 10 further comprising selecting a cell comprising an altered target nucleic acid having a desired activity.

13. (Amended) A method according to claim 10 further comprising selecting a cell comprising an altered target nucleic acid and having a desired phenotype.

14. (Amended) A method according to claim 11 further comprising secreting said library of variant amino acid sequences.

15. (Amended) A method according to claim 10 wherein said recombinase is removed prior to said introducing.

16. (Amended) A method according to claim 29 wherein said cell is eukaryotic.

17. (Amended) A method according to claim 29 wherein said cell is procaryotic.

18. (Amended) A method according to claim 1, 2, 3, 4, 5, 6, 7, 8, 25, 26, 27, or 28 wherein said targeting polynucleotides are coated with said recombinase.

19. (Amended) A method according to claim 1, 2, 3, 4, 5, 6, 7, 8, 25, 26, 27, or 28 wherein said recombinase is a species of prokaryotic recombinase.

20. (Amended) A method according to claim 1, 2, 3, 4, 5, 6, 7, 8, 25, 26, 27, or 28 wherein said recombinase is a species of eukaryotic recombinase.

21. (Amended) A method according to claim 11, wherein said variant polypeptides comprise a plurality of amino acid substitutions.

22. (Amended) A method according to claim 1, 2, 3, 4, 5, 6, 7, 8, 25, 26, 27, or 28 wherein at least one of said targeting polynucleotides further comprises a chemical substituent.

23. (Amended) A method according to claim 1, 2, 3, 4, 5, 6, 7, 8, 25, 26, 27, or 28 wherein said target amino acid comprises a complementary determining region.

24. (Amended) A method according to claim 1, 2, 3, 4, 5, 6, 7, 8, 25, 26, 27, or 28 wherein said target nucleic acid comprises an expression vector.

Please add the following new claims:

25. (New) A method according to claim 1, further comprising:

contacting all or part of said first library of altered nucleic acids with a second plurality of pairs of single-stranded targeting polynucleotides which are substantially complementary to each other and are not substantially complementary to said first plurality of polynucleotides, wherein each said targeting polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a second predetermined sequence of said target nucleic acid encoding a second domain of said polypeptide, said second plurality of pairs comprising a second library of nucleic acids having mismatches between said second targeting polynucleotides and said second predetermined sequence, to form a second library of altered target nucleic acids.

26. (New) A method according to claim 3 further comprising:

d) contacting said first recombination intermediate with a second pair of single-stranded targeting polynucleotides which are substantially complementary to each other and are not substantially complementary to said first pair of polynucleotides, wherein each targeting polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a second predetermined sequence of said target nucleic acid encoding a second domain of said polypeptide, to form a second recombination intermediate, wherein said contacting of step b) is of said second recombination intermediate with said nuclease.

27. (New) A method according to claim 5 further comprising:

c) contacting all or part of said first library of altered target nucleic acids with at least one

recombinase and a second plurality of pairs of single-stranded targeting polynucleotides which are substantially complementary to each other and are not substantially complementary to said first plurality of polynucleotides, wherein each polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a second preselected sequence of said target nucleic acid, said second plurality of pairs comprising a second library of nucleic acids having mismatches between said targeting polynucleotides and said second preselected sequence, to evolve a second library of altered target nucleic acids,

wherein said repeating is on said second library of altered target nucleic acids.

28. (New) A method according to claim 7 further comprising:

c) contacting all or part of said first library of altered target nucleic acids with at least one recombinase and a second plurality of pairs of single-stranded targeting polynucleotides which are substantially complementary to each other and are not substantially complementary to said first plurality of polynucleotides, wherein each said polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a second preselected sequence of said target nucleic acid, said second plurality of pairs comprising a second library of nucleic acids having mismatches between said targeting polynucleotides and said second preselected sequence, to evolve a second library of altered target nucleic acids,

wherein said repeating is on said second library of altered target nucleic acids.

29. (New) A method according to claim 1, 2, 3, 4, 5, 6, 7, 8, 25, 26, 27, or 28 further comprising contacting said recombination intermediate with a recombination proficient cell.

REMARKS

Claims 1-29 are pending. Support for the amendments is found in the claims as filed, and in particular as follows. Further support for the amendments to claims 1-2 can be found at p.4,

Serial No.: 09/886,171
Filing Date: June 20, 2001

lines 1-2. Support for claim 14 can be found at p. 35, line 21. Support for new claims 25-28 can be found in claims 1-8 as filed, and additionally at page 27, lines 25-27 and p. 28, lines 27-29. Support for new claim 29 can be found at p. 6, line 22. Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "MARKED UP VERSION TO SHOW CHANGES MADE." Also attached hereto is a copy of the pending claims, for the Examiner's convenience.

The present case is a continuation of U.S.S.N. 09/373,347.

Enclosed herewith is a separate amendment ("Amendment Re Sequence Listing") which seeks to bring the application into compliance with 37 C.F.R. § 1.821-1.825.

Although no fees are believed to be due at this time, the Commissioner is authorized to charge any fees including extension fees or other relief which may be required, or credit any overpayment to Deposit Account No. 06-1300 (Our Order No. A-66914-2/RFT/AMS).

Applicants respectfully submit that the claims are in condition for allowance and an early notification of such is solicited. If, upon review, the Examiner feels there are additional outstanding issues, the Examiner is invited to call the undersigned attorney.

Respectfully submitted,

FLEHR HOHBACH TEST
ALBRITTON & HERBERT LLP

Date: September 20, 2001

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Serial No.: 09/886,171
Filing Date: June 20, 2001

MARKED UP VERSION TO SHOW CHANGES MADE

The paragraph at page 1 beginning at line 4 has been amended as follows:

--This application is a [continuing application of United States Application No. 60/096,330 filed August 12, 1998, pending and United States Application No. 09,133,934 filed August 14, 1998, pending] continuation of U.S. Application No. 09/373,347, filed August 12, 1999, which is a continuation-in-part of U.S. Application No. 09/133,934, filed August 14, 1998, now U.S. Patent No. 6,074,853 and which claims the benefit of U.S. Provisional Application No. 60/096,330, filed August 12, 1998.--

The paragraph at page 37 beginning at line 11 has been amended as follows:

To evolve the scFV to higher affinities, probes are synthesized to target CDRs in the light and heavy chains. Each probe has sequences that are dengerate for corresponding CDRs, but homologous to the frame-work regions for homology clamping (Figure [8] 5). The probes are combined with RecA to form nucleoprotein filament as described in Example 1. The filament are hybridized to purified scFv phagemid DNA to produce a hybrid complex. Complexes are transformed into recombination proficient *E. coli* strain (e.g. BB4) to allow strand exchange. The bacteria are also transformed with helper phage to assemble and package the scFv phagement containing mutagenized or evolved CDR regions.

The Claims have been amended as follows:

1. (Amended) A method of domain specific gene evolution of a target nucleic acid [sequence] encoding an amino acid sequence of interest, said method comprising:

contacting a target nucleic acid with a recombinase and a first [providing a] plurality of pairs of single-stranded targeting polynucleotides which are substantially complementary to each other, wherein [and] each said targeting polynucleotide comprises [comprising] a homology clamp that substantially corresponds to or is substantially complementary to a first predetermined sequence of said target nucleic acid [sequence] encoding a first domain of a polypeptide [said protein], said first plurality of pairs comprising a first library of nucleic acids having mismatches between said targeting polynucleotides and said first predetermined sequence [and a recombinase], to form a first library of altered target nucleic acids [acid sequences]; and repeating said contacting on said library of altered nucleic acids.

2. (Amended) A method according to claim 1, further comprising:

[simultaneously or successively providing] contacting said target nucleic acid with a second plurality of pairs of single-stranded targeting polynucleotides[,] which are substantially complementary to each other and are not substantially complementary to said first plurality of polynucleotides, wherein each said targeting polynucleotide comprises [and each comprising] a [second] homology clamp[,] that substantially

corresponds to or is substantially complementary to a second predetermined sequence of said target nucleic acid [sequence] encoding a second domain of said polypeptide [protein], said second plurality of pairs comprising a second library of nucleic acids having mismatches between said second targeting polynucleotides and said second predetermined sequence [and a recombinase], to form a second library of altered target nucleic acids [acid sequences].

3. (Amended) A method of domain specific gene evolution comprising:
a) contacting [combining] a target nucleic acid encoding a polypeptide [an amino acid sequence] of interest with a recombinase and a first pair of single-stranded targeting polynucleotides which are substantially complementary to each other, wherein each said targeting polynucleotide comprises [and each comprising] a homology clamp that substantially corresponds to or is substantially complementary to a first predetermined sequence of said nucleic acid encoding a first domain of said polypeptide [protein], [and a recombinase] to form a first recombination intermediate;
b) contacting said recombination intermediate with a [single-strand exonuclease or junction-specific] nuclease to form a nicked or open ended target nucleic acid; and
c) reassembling and recombining said nicked or open ended target nucleic acid to evolve [produce] a first library of altered target nucleic acids.

4. (Amended) A method according to claim 3 further comprising:
d) [simultaneously or successively] combining said target nucleic acid [encoding said amino acid sequence of interest] with a second pair of single-stranded targeting polynucleotides which are substantially complementary to each other and are not substantially complementary to said first pair of polynucleotides, wherein each targeting polynucleotide comprises [and each comprising] a homology clamp that substantially corresponds to or is substantially complementary to a second predetermined sequence of said target nucleic acid encoding a second domain of said polypeptide [protein], [and a recombinase] to form a second recombination intermediate[;], wherein said contacting of step b) is of said second recombination intermediate with said nuclease
[e) contacting said intermediate with a single-strand exonuclease or junction-specific nuclease to form a nicked or open ended target nucleic acid; and
f) reassembling and recombining said nicked or open ended target nucleic acid to produce a library of altered target nucleic acids].

5. (Amended) A method of generating a library of altered [pool of variant] nucleic acid sequences of a pre-selected target nucleic acid sequence in an extrachromosomal sequence, said method comprising:
a) contacting an extrachromosomal nucleic acid comprising a target nucleic acid sequence with [adding to said extrachromosomal sequence] at least one recombinase and a first plurality of pairs of single-stranded targeting polynucleotides which are

substantially complementary to each other, wherein each said targeting polynucleotide comprises [and each comprising] a homology clamp that substantially corresponds to or is substantially complementary to a first preselected [target nucleic acid] sequence of said target nucleic acid, said first plurality of pairs comprising a first library of nucleic acids having mismatches between said targeting polynucleotides and said [target nucleic acid sequence] first preselected sequence, to evolve [form]a first library of altered target nucleic acids [extrachromosomal sequences]; and

b) repeating step a) on said library of altered target nucleic acids [extrachromosomal sequences].

6. (Amended) A method according to claim 5 further comprising:

c) adding [simultaneously or successively] to said extrachromosomal nucleic acid [sequence at least one recombinase and] a second plurality of pairs of single-stranded targeting polynucleotides which are substantially complementary to each other and are not substantially complementary to said first plurality of polynucleotides, wherein each said targeting polynucleotide comprises [and each comprising] a homology clamp that substantially corresponds to or is substantially complementary to a second preselected [target nucleic acid] sequence of said target nucleic acid, said second plurality of pairs comprising a second library of nucleic acids having mismatches between said targeting polynucleotide and said second preselected [target nucleic acid] sequence, to evolve [form] a second library of altered target nucleic acids, [extrachromosomal sequences;] wherein said repeating is on said second library of altered target nucleic acids

[e) repeating step d) on said library of altered extrachromosomal sequences].

7. (Amended) A method of generating a library of altered [pool of variant] nucleic acids [acid sequences] of a pre-selected target nucleic acid [sequence] in a[n] chromosomal sequence, said method comprising:

a) contacting a [adding to said] chromosomal nucleic acid [sequence] comprising a target nucleic acid with at least one recombinase and a first plurality of pairs of single-stranded targeting polynucleotides which are substantially complementary to each other, wherein each said polynucleotide comprises [and each comprising] a homology clamp that substantially corresponds to or is substantially complementary to a first preselected [target nucleic acid] sequence of said target nucleic acid, said plurality of pairs comprising a first library of nucleic acids having mismatches between said targeting polynucleotides and said first preselected [target nucleic acid] sequence, to form [evolve] a first library of altered target nucleic acids [chromosomal sequences]; and

b) repeating step a) on said library of altered target nucleic acids [extrachromosomal sequences].

8. (Amended) A method according to claim 7 further comprising:

c) adding [simultaneously or successively] to said chromosomal nucleic acid [sequence at least one recombinase and] a second plurality of pairs of single-stranded targeting

polynucleotides which are substantially complementary to each other and are not substantially complementary to said first plurality of polynucleotides, wherein each said polynucleotide comprises [and each comprising] a homology clamp that substantially corresponds to or is substantially complementary to a second preselected [target nucleic acid] sequence of said target nucleic acid, said second plurality of pairs comprising a second library of nucleic acids having mismatches between said targeting polynucleotides and said second preselected [target nucleic acid] sequence, to evolve [form] a second library of altered target nucleic acids, [chromosomal sequences; and]

wherein said repeating is on said second library of altered target nucleic acids

[e) repeating step d) on said library of altered chromosomal sequences].

9. (Amended) A method according to any one of claims 1, 2, 3, [or] 4, 25, 26, 27, and 28 further comprising repeating said method on said library of altered target nucleic acids.

10. (Amended) A method according to any one of claims 1, 2, 3, 4, 5, 6, 7, [or] 8, 25, 26, 27, and 28 further comprising introducing said library of altered target nucleic acids into cells to form a cellular library comprising variant nucleic acid sequences.

11. (Amended) A method according to claim 10 further comprising expressing said library of altered target nucleic acids [acid sequences] to generate a library [pool] of variant polypeptides [amino acid sequences].

12. (Amended) A method according to claim 10 [or 11] further comprising selecting a cell comprising an altered target nucleic acid [sequence] having a desired activity.

13. (Amended) A method according to claim 10 [or 11] further comprising selecting a cell comprising an altered target nucleic acid [sequence] and having a desired phenotype.

14. (Amended) A method according to claim 11 further comprising secreting said library [pool] of variant amino acid sequences.

15. (Amended) A method according to claim 10[, 11, 12, or 13] wherein said recombinase is removed prior to said introducing.

16. (Amended) A method according to claim 29 [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15] wherein said cell is [cells are] eukaryotic.

17. (Amended) A method according to claim 29 [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16] wherein said cell is [cells are] procaryotic.

18. (Amended) A method according to claim 1, 2, 3, 4, 5, 6, 7, 8, [9, 10, 11, 12, 13, 14, 15, 16, or 17] 25, 26, 27, or 28 wherein said targeting polynucleotides are coated with said recombinase.

19. (Amended) A method according to claim 1, 2, 3, 4, 5, 6, 7, 8, [9, 10, 11, 12, 13, 14, 15, 16, 17, or 18] 25, 26, 27, or 28 wherein said recombinase is a species of prokaryotic recombinase.

20. (Amended) A method according to claim 1, 2, 3, 4, 5, 6, 7, 8, [9, 10, 11, 12, 13, 14, 15, 16, 17, or 18] 25, 26, 27, or 28 wherein said recombinase is a species of eukaryotic recombinase.

21. (Amended) A method according to claim 11, wherein said [the] variant polypeptides [amino acid sequences] comprise a plurality of amino acid substitutions.

22. (Amended) A method according to claim 1, 2, 3, 4, 5, 6, 7, 8, [9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21] 25, 26, 27, or 28 wherein at least one of said targeting polynucleotides [complementary single stranded nucleic acids] further comprises a chemical substituent.

23. (Amended) A method according to claim 1, 2, 3, 4, 5, 6, 7, 8, [9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or 22] 25, 26, 27, or 28 wherein said [the] target amino acid [sequence] comprises a complementary determining region.

24. (Amended) A method according to claim 1, 2, 3, 4, 5, 6, 7, 8, [9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, or 23] 25, 26, 27, or 28 wherein said target nucleic acid comprises an expression vector.

Serial No.: 09/886,171
Filing Date: June 20, 2001

Pending Claims:

1. (Amended) A method of domain specific gene evolution of a target nucleic acid encoding an amino acid sequence of interest, said method comprising:
contacting a target nucleic acid with a recombinase and a first plurality of pairs of single-stranded targeting polynucleotides which are substantially complementary to each other, wherein each said targeting polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a first predetermined sequence of said target nucleic acid encoding a first domain of a polypeptide, said first plurality of pairs comprising a first library of nucleic acids having mismatches between said targeting polynucleotides and said first predetermined sequence, to form a first library of altered target nucleic acids; and repeating said contacting on said library of altered nucleic acids.

2. (Amended) A method according to claim 1, further comprising:
contacting said target nucleic acid with a second plurality of pairs of single-stranded targeting polynucleotides which are substantially complementary to each other and are not substantially complementary to said first plurality of polynucleotides, wherein each said targeting polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a second predetermined sequence of said target nucleic acid encoding a second domain of said polypeptide, said second plurality of pairs comprising a second library of nucleic acids having mismatches between said second targeting polynucleotides and said second predetermined sequence, to form a second library of altered target nucleic acids.

3. (Amended) A method of domain specific gene evolution comprising:
a) contacting a target nucleic acid encoding a polypeptide of interest with a recombinase and a first pair of single-stranded targeting polynucleotides which are substantially complementary to each other, wherein each said targeting polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a first predetermined sequence of said nucleic acid encoding a first domain of said polypeptide, to form a first recombination intermediate;
b) contacting said recombination intermediate with a nuclease to form a nicked or open ended target nucleic acid; and
c) reassembling and recombining said nicked or open ended target nucleic acid to evolve a first library of altered target nucleic acids.

4. (Amended) A method according to claim 3 further comprising:
d) combining said target nucleic acid with a second pair of single-stranded targeting polynucleotides which are substantially complementary to each other and are not substantially complementary to said first pair of polynucleotides, wherein each targeting polynucleotide comprises a homology clamp that substantially corresponds to or is

substantially complementary to a second predetermined sequence of said target nucleic acid encoding a second domain of said polypeptide, to form a second recombination intermediate, wherein said contacting of step b) is of said second recombination intermediate with said nuclease.

5. (Amended) A method of generating a library of altered nucleic acid sequences of a pre-selected target nucleic acid sequence in an extrachromosomal sequence, said method comprising:

a) contacting an extrachromosomal nucleic acid comprising a target nucleic acid sequence with at least one recombinase and a first plurality of pairs of single-stranded targeting polynucleotides which are substantially complementary to each other, wherein each said targeting polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a first preselected sequence of said target nucleic acid, said first plurality of pairs comprising a first library of nucleic acids having mismatches between said targeting polynucleotides and said first preselected sequence, to evolve a first library of altered target nucleic acids; and
b) repeating step a) on said library of altered target nucleic acids.

6. (Amended) A method according to claim 5 further comprising:
c) adding to said extrachromosomal nucleic acid a second plurality of pairs of single-stranded targeting polynucleotides which are substantially complementary to each other and are not substantially complementary to said first plurality of polynucleotides, wherein each said targeting polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a second preselected sequence of said target nucleic acid, said second plurality of pairs comprising a second library of nucleic acids having mismatches between said targeting polynucleotide and said second preselected sequence, to evolve a second library of altered target nucleic acids,
wherein said repeating is on said second library of altered target nucleic acids.

7. (Amended) A method of generating a library of altered nucleic acids of a pre-selected target nucleic acid in a chromosomal sequence, said method comprising:
a) contacting a chromosomal nucleic acid comprising a target nucleic acid with at least one recombinase and a first plurality of pairs of single-stranded targeting polynucleotides which are substantially complementary to each other, wherein each said polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a first preselected sequence of said target nucleic acid, said plurality of pairs comprising a first library of nucleic acids having mismatches between said targeting polynucleotides and said first preselected sequence, to form a first library of altered target nucleic acids; and
b) repeating step a) on said library of altered target nucleic acids.

8. (Amended) A method according to claim 7 further comprising:

c) adding to said chromosomal nucleic acid a second plurality of pairs of single-stranded targeting polynucleotides which are substantially complementary to each other and are not substantially complementary to said first plurality of polynucleotides, wherein each said polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a second preselected sequence of said target nucleic acid, said second plurality of pairs comprising a second library of nucleic acids having mismatches between said targeting polynucleotides and said second preselected sequence, to evolve a second library of altered target nucleic acids,

wherein said repeating is on said second library of altered target nucleic acids.

9. (Amended) A method according to any one of claims 1, 2, 3, 4, 25, 26, 27, and 28 further comprising repeating said method on said library of altered target nucleic acids.

10. (Amended) A method according to any one of claims 1, 2, 3, 4, 5, 6, 7, 8, 25, 26, 27, and 28 further comprising introducing said library of altered target nucleic acids into cells to form a cellular library comprising variant nucleic acid sequences.

11. (Amended) A method according to claim 10 further comprising expressing said library of altered target nucleic acids to generate a library of variant polypeptides.

12. (Amended) A method according to claim 10 further comprising selecting a cell comprising an altered target nucleic acid having a desired activity.

13. (Amended) A method according to claim 10 further comprising selecting a cell comprising an altered target nucleic acid and having a desired phenotype.

14. (Amended) A method according to claim 11 further comprising secreting said library of variant amino acid sequences.

15. (Amended) A method according to claim 10 wherein said recombinase is removed prior to said introducing.

16. (Amended) A method according to claim 29 wherein said cell is eukaryotic.

17. (Amended) A method according to claim 29 wherein said cell is procaryotic.

18. (Amended) A method according to claim 1, 2, 3, 4, 5, 6, 7, 8, 25, 26, 27, or 28 wherein said targeting polynucleotides are coated with said recombinase.

19. (Amended) A method according to claim 1, 2, 3, 4, 5, 6, 7, 8, 25, 26, 27, or 28 wherein said recombinase is a species of prokaryotic recombinase.

20. (Amended) A method according to claim 1, 2, 3, 4, 5, 6, 7, 8, 25, 26, 27, or 28 wherein said recombinase is a species of eukaryotic recombinase.

21. (Amended) A method according to claim 11, wherein said variant polypeptides comprise a plurality of amino acid substitutions.

22. (Amended) A method according to claim 1, 2, 3, 4, 5, 6, 7, 8, 25, 26, 27, or 28 wherein at least one of said targeting polynucleotides further comprises a chemical substituent.

23. (Amended) A method according to claim 1, 2, 3, 4, 5, 6, 7, 8, 25, 26, 27, or 28 wherein said target amino acid comprises a complementary determining region.

24. (Amended) A method according to claim 1, 2, 3, 4, 5, 6, 7, 8, 25, 26, 27, or 28 wherein said target nucleic acid comprises an expression vector.

25. (New) A method according to claim 1, further comprising:
contacting all or part of said first library of altered nucleic acids with a second plurality of pairs of single-stranded targeting polynucleotides which are substantially complementary to each other and are not substantially complementary to said first plurality of polynucleotides, wherein each said targeting polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a second predetermined sequence of said target nucleic acid encoding a second domain of said polypeptide, said second plurality of pairs comprising a second library of nucleic acids having mismatches between said second targeting polynucleotides and said second predetermined sequence, to form a second library of altered target nucleic acids.

26. (New) A method according to claim 3 further comprising:
d) contacting said first recombination intermediate with a second pair of single-stranded targeting polynucleotides which are substantially complementary to each other and are not substantially complementary to said first pair of polynucleotides, wherein each targeting polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a second predetermined sequence of said target nucleic acid encoding a second domain of said polypeptide, to form a second recombination intermediate, wherein said contacting of step b) is of said second recombination intermediate with said nuclease.

27. (New) A method according to claim 5 further comprising:
c) contacting all or part of said first library of altered target nucleic acids with at least one recombinase and a second plurality of pairs of single-stranded targeting polynucleotides which are substantially complementary to each other and are not substantially complementary to said first plurality of polynucleotides, wherein each polynucleotide comprises a homology clamp that substantially corresponds to or is substantially

complementary to a second preselected sequence of said target nucleic acid, said second plurality of pairs comprising a second library of nucleic acids having mismatches between said targeting polynucleotides and said second preselected sequence, to evolve a second library of altered target nucleic acids,

wherein said repeating is on said second library of altered target nucleic acids.

28. (New) A method according to claim 7 further comprising:

c) contacting all or part of said first library of altered target nucleic acids with at least one recombinase and a second plurality of pairs of single-stranded targeting polynucleotides which are substantially complementary to each other and are not substantially complementary to said first plurality of polynucleotides, wherein each said polynucleotide comprises a homology clamp that substantially corresponds to or is substantially complementary to a second preselected sequence of said target nucleic acid, said second plurality of pairs comprising a second library of nucleic acids having mismatches between said targeting polynucleotides and said second preselected sequence, to evolve a second library of altered target nucleic acids,

wherein said repeating is on said second library of altered target nucleic acids.

29. (New) A method according to claim 1, 2, 3, 4, 5, 6, 7, 8, 25, 26, 27, or 28 further comprising contacting said recombination intermediate with a recombination proficient cell.

Attorney Docket No.: A-66914-2/RFT/BTC/AMS

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

ZARLING et al..

Serial No. 09/886,171

Filed: June 20, 2001

For: DOMAIN SPECIFIC GENE EVOLUTION

Examiner: UNKNOWN

Group Art Unit:

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as First Class Mail in an envelope addressed to: Assistant Commissioner of Patents, Washington, DC 20231 on Sept. 20, 2001.

Signed: Maria Ciganovich

PRELIMINARY AMENDMENT RE SEQUENCE LISTING

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

This Amendment is in anticipation of a Notice to Comply with Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures.

Although no fee is believed to be due at this time, the Commissioner is authorized to charge any fees including extension fees or other relief which may be required, or credit any overpayment to Deposit Account No. 06-1300 (Our Order No. A-66914-2/RFT/BTC/AMS).

Please amend the application as follows to comply with requirements for patent applications containing nucleotide sequence and/or amino acid sequence disclosures in adherence with rules 37 C.F.R. § 1.821-1.825:

Serial No.: 09/886,171
Filed: June 20, 2001

IN THE SPECIFICATION:

Please replace the paragraph beginning at page 5, line 4, with the following rewritten paragraph:

—Figure 5 (SEQ ID NO:1-6) depicts targeting polynucletides for evolving CDR region of scFv to Botulinum neurotoxin. —

On page 39, immediately preceding the claims, please insert the enclosed text entitled "SEQUENCE LISTING".

REMARKS

The specification has been amended to include a Sequence Listing and proper reference to the sequences therein. Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with markings to show changes made."

Entry of this amendment is respectfully requested. The amendments are made in adherence with 37 C.F.R. § 1.821-1.825. This amendment is accompanied by a floppy disk containing the above named sequence, SEQUENCE ID NUMBERS 1-6 in computer readable form, and a paper copy of the sequence information. The computer readable sequence listing was prepared through use of the software program "PatentIn" provided by the PTO. The information contained in the computer readable disk is identical to that of the paper copy. This amendment contains no new matter. Applicant submits that this amendment, the accompanying computer readable sequence listing, and the paper copy thereof serve to place this application in a condition of adherence to the rules 37 C.F.R. § 1.821-1.825.

Serial No.: 09/886,171
Filed: June 20, 2001

Please direct any calls in connection with this application to the undersigned at (415) 781-1989.

Respectfully submitted,

FLEHR HOHBACH TEST
ALBRITTON & HERBERT LLP

Dated: September 20, 2001

Richard F. Trecartin, Reg. No. 31,801

Four Embarcadero Center
Suite 3400
San Francisco, CA 94111-4187
Telephone: (415) 781-1989

Serial No.: 09/886,171
Filed: June 20, 2001

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

Paragraph beginning at page 5, line 4, has been amended as follows:

–Figure 5 (SEQ ID NO:1-6) depicts targeting polynucletides for evolving CDR region of scFv to Botulinum neurotoxin. –

On page 39, immediately preceding the claims, the enclosed Sequence Listing was added to the text.

PATENT
Attorney Docket No.: A-66914-1/RFT/BTC/AMS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

ZARLING et al.

Serial No. 09/373,347

Filed: August 12, 1999

For: DOMAIN SPECIFIC GENE EVOLUTION

Examiner: LOEB, B.

Group Art Unit: 1636

"EXPRESS MAIL" MAILING LABEL NUMBER **EL758643351US**
DATE OF DEPOSIT **June 20, 2001**
I HEREBY CERTIFY THAT THIS PAPER OR FEE IS BEING DEPOSITED WITH THE UNITED STATES POSTAL SERVICE "EXPRESS MAIL POST OFFICE TO ADDRESSEE" SERVICE UNDER 37 CFR 1.10 ON THE DATE INDICATED ABOVE AND IS ADDRESSED TO: ASSISTANT COMMISSIONER FOR PATENTS, WASHINGTON, DC 20231.
TYPED NAME **Darryl Kriner**
SIGNED

AMENDMENT RE SEQUENCE LISTING

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

This Amendment is in response to the Office Action mailed December 20, 2000. The present Amendment is submitted to comply with requirements for patent applications containing nucleotide sequence and/or amino acid sequence disclosures.

Please amend the application as follows to comply with requirements for patent applications containing nucleotide sequence and/or amino acid sequence disclosures in adherence with rules 37 C.F.R. § 1.821-1.825:

Serial No.: 09/373,347
Filed: August 12, 1999

IN THE SPECIFICATION:

Please replace the paragraph beginning at page 5, line 4, with the following rewritten paragraph:

—Figure 5 (SEQ ID NO:1-6) depicts targeting polynucletides for evolving CDR region of scFv to Botulinum neurotoxin. —

On page 39, immediately preceding the claims, please insert the enclosed text entitled "SEQUENCE LISTING".

REMARKS

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with markings to show changes made."

Entry of this amendment is respectfully requested. The amendments are made in adherence with 37 C.F.R. § 1.821-1.825. This amendment is accompanied by a floppy disc containing the above named sequence, SEQUENCE ID NUMBERS 1-6 in computer readable form, and a paper copy of the sequence information. The computer readable sequence listing was prepared through use of the software program "PatentIn" provided by the PTO. The information contained in the computer readable disc is identical to that of the paper copy. This amendment contains no new matter. Applicant submits that this amendment, the accompanying computer readable sequence listing,

and the paper copy thereof serve to place this application in a condition of adherence to the rules 37 C.F.R. § 1.821-1.825.

The Commissioner is authorized to charge any additional fees including extension fees or other relief which may be required, or credit any overpayment to Deposit Account No. 06-1300 (Our Order No. A-66914-1/RFT/BTC/AMS).

Please direct any calls in connection with this application to the undersigned at (415) 781-1989.

Respectfully submitted,

FLEHR HOHBACH TEST
ALBRITTON & HERBERT LLP

Dated: 6/20/01

Richard F. Trecartin, Reg. No. 31,801

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Telephone: (415) 781-1989

Serial No.: 09/373,347
Filed: August 12, 1999

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

Paragraph beginning at page 5, line 4, has been amended as follows:

—Figure 5 (SEQ ID NO:1-6) depicts targeting polynucletides for evolving CDR region of scFv to Botulinum neurotoxin. —

On page 39, immediately preceding the claims, the enclosed Sequence Listing was added to the text.